



## Heavy Metals in Sludge Produced from UASB Treatment Plant at Mirzapur, India

Vijai Krishna\*, Anil Kumar Pandey and Pankaj Kumar Gupta

Department of Environmental Sciences (Environmental Technology), RGSC, (BHU), Mirzapur, India

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### ABSTRACT

In Mirzapur (U.P.), a power-starved district, the UASB (Upflow Anaerobic Sludge Blanket) technique was adopted. Almost all of the available technologies do not treat heavy metals, so, is the case with the UASB also. The present study is to assess how much heavy metal can get accumulated in plant tissues in different species. The result of the present study was that the concentration of Pb(1106.31)>Zn(221.45)>Cd(49.26)>Hg(23.37) mg/Kg in the sludge while the concentration of Zn(93.35)>Pb(52.00)>Hg(16.93)>Cd(1.53) mg/Kg in the soil. When the sludge was mixed with the soil the trend got changed and the trend was Pb(596.36)>Zn(219.86)>Cd(24.70)>Hg(22.63) mg/Kg. Three different species that were chosen for the study were *Basella Alba* (Spinach), *Solanum Lycopersicum* (Tomato) & *Brassica Juncea* (Mustard). The trend of accumulation of studied heavy metals in the *Brassica Juncea* (Mustard) was Zn(85.33)>Pb(25.88)>Hg(11.23)>Cd(0.99) mg/Kg. In *Solanum lycopersicum* (Tomato) the trend was Pb(231.11)>Zn(108.72)>Hg(12.43)>Cd(9.41) mg/Kg and in *Basella alba* (Spinach) was Zn(103.81)>Pb(83.90)>Hg(10.78)>Cd(4.18) mg/Kg. Overall the study reveals that the accumulation of heavy metals takes place in plants grown in soil mixed with sewage sludge. The reduction in the concentration of Pb, Cd, Hg and Zn in sludge mixed with soil after the harvesting of plant in case of *Solanum lycopersicum* were 39.38%, 47.93%, 6.18% and 49.89% respectively; while in case of *Basella alba* these were 25.23%, 57.53%, 71.58% and 49.16% respectively; and in case of *Brassica Juncea* these reduction were 25.86%, 60.80%, 70.96% and 49.04% respectively.

**KEYWORDS:** Pollution, Water Treatment, Sludge, Fertilizers, Agriculture.

### INTRODUCTION

The waste water treatment plants mostly focus on the removal of suspended solids and organic matter. In most of the sewage treatment plants, there are no provisions for removal of heavy metals so there is every possibility that the sludge or the treated water released may contain heavy metals. The sludge from the UASB plant is no exception as here also there is no provision of treatment of heavy metals. This sludge is being sold as fertilizer to farmers who mostly grow vegetables in such soils which are amended through sludge. The vegetables grown on such sludge amended soils are likely to accumulate appreciable quantities of heavy metals in them through plant uptake. Under such circumstances the health of people consuming such vegetables is at stake, therefore it is important to know whether the vegetable crops accumulate heavy metals in quantities sufficient to cause harm to the human population or not. It is in light of these facts that the present study has been carried out. Heavy metals apply to the metals and

\* Corresponding Author, Email: vijaikrishna@bhu.ac.in

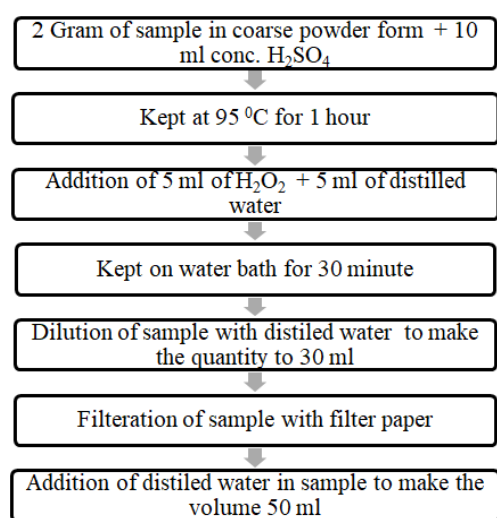
metalloids with atomic density more than  $4\text{g/cm}^3$  (Hawkes, 1997). Scientists are working to assess the effects of heavy metals on plants (Reeves and Baker, 2000; Fernandes and Henriques, 1991). Metal ions, through Haber–Weiss reaction induce oxidative modifications of free amino acids and proteins (Stadtman, 1993). Metal binding to the cell nucleus causes genotoxicity (Kasprzak, 1995). Metal-mediated oxygen may cause the genotoxicity in plants (Cunningham, 1997). Sewage sludge is rich in organic matter and nutrients, so it may be utilized in agriculture as manure but restricted due to excessive heavy metals concentration (Jakubus & Czekala, 2001). Sewage sludge from urban wastewater treatment stations is likely to contain high levels of Ni, Pb, Cu, Zn, Mn and Cd (Lixandru et al., 2010). An account of accumulation of heavy metals in sewage watered soils was given (Chen et al., 2009). Heavy metals in sludge can accumulate on biological debris (Jaroslaw & Barbara, 2012). Heavy metals may present in several chemical forms. Studies on sludge amended soils indicate for higher concentration of heavy metal in topsoil. Increase of heavy metals in vegetables has been studied (Arora et al., 2008). Municipal sewage sludge (MSS) used in agriculture might impact quality of crop and health of people (George et al., 2011) but use of sludge in soils can increase the fertility of soil and production (Ebrahim et al., 2018). Sewage sludge may holds nutrients like nitrogen, phosphorus and organic matter (Tontti et al., 2017). Use of sludge to soil cultivates physicochemical properties and boosts productivity but may contaminate the soil plastic too (Bogusz et al., 2017). So, use of sludge permits recycling of organic matter and nutrients (Kominko et al., 2019). Mixing composts to soil may modify physicochemical properties and increase the growth due to the presence of great organic matter and nutrients (Liu et al., 2019). There are substantial associations between concentrations of heavy metal and activities of enzymes in soil (Liu et al., 2020). Using compost of sewage sludge into soil with planting suitable trees could possibly deliver an operative way for sludge disposal and cut the use of fertilizer (Chu et al., 2017). Use of sewage sludge can enhance the pH of acidic soils and the fertility mainly in respect of N and P (Samara et al., 2017). It is possible to modify soil contaminated with metal by the application of sewage sludge biochars, because it reduces the mobility of metals and so the risk of leaching (Zhou et al., 2017).

## MATERIALS & METHODS

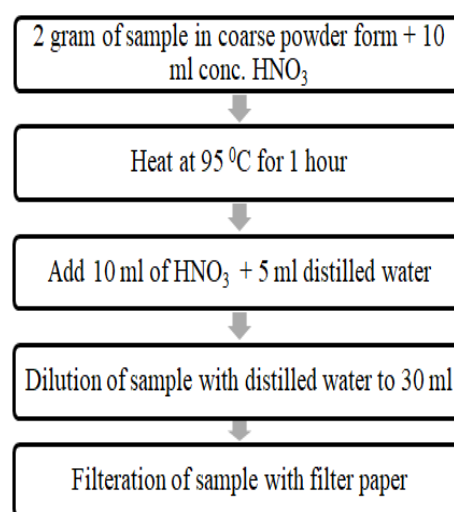
Initially three plots of dimension  $40\times 40$  cm. were prepared and each plot was filed with 4.5 kilograms of sludge which was collected from the UASB Mirzapur. Three species of plants viz. Mustard, Spinach & Tomato were grown separately in the three plots. The plots were irrigated with distilled water when needed. The plants started dying after 15 days and hence the experiment was terminated after 15 days. However, the plants from each plot were collected, dried and were examined for heavy metals which might have accumulated in the plant tissue owing to their uptake by atomic absorption spectrophotometer (AAS) (SHIMADZU AA6300). The sludge from each plot was also collected to assess the extent of heavy metals removed from the sludge. The second stage of the experiment was carried out using a mixture of 50% soil and 50% sludge. Nine plots of dimension  $40\times 40$  cm. each was prepared and filed with a mixture of soil and sludge. This time, three plants of each species were grown in separate nine plots. The plants were watered using distilled water when needed. This time the plants survived but for the sake of comparison, the trial was come to an end after 15 days and the plants from each plot were collected, dried and analyzed. The sludge and soil mixture was also assessed to examine the probable reduction in the concentration of the heavy metals. After completing above experiments, 15 samples were obtained for their analysis. The methods or preparation of samples is given in figure 1 and figure 2.

## RESULTS & DISCUSSION

Shoot and root lengths of plants of each plot grown on sludge were measured after fifteen days and their average was calculated. The Same procedure was applied for the plants which were grown in sludge mixed with soil in ratio 1:1. The data of experiment is given in table 1. It has been observed (Angino et al., 1970) that enzymes occurred in detergents may contain heavy metals like Fe, Mn, Cr, Co, Zn, Sr and B in traces. Urban storm water runoff is a source of pollutants. (Bradford, 1997). Heavy metal pollution occurs more in nearby mining areas. (Peplow, 1999). Human actions increases the heavy metal availability to environment, which interfere the physiological process of the plants like photosynthesis, water and nutrient uptake etc. The collection of heavy metal is affected by soil temperature, soil pH, soil moisture, types of roots, types of leaves, types of plants, its physiological processes etc. (Yamamoto and Kozlowski, 1987). It has been observed that increase in pH and reduction in redox potential, results in lower availability of heavy metals to plants (Misra and Mani). Heavy metals levels in plants vary widely (Wong, 1996). These heavy metals are concerned as pollutants because they pose toxic effects both acute and chronic on plants (Nagajyoti et al., 2010). Heavy metals from sludge can enter into food chain through absorption of water (Clarke et al., 2016). So, the direct application of municipal sewage sludge to agricultural is a controversial practice for a longer period of time (Hei et al., 2016). Beneficial effect of the use of sewage sludge has been seen in respect of plant characteristics and the overall yield of carrot (Nahar & Hossen, 2020). Sewage composts may be used for soilless cultivation and is an alternative of peat, even contains heavy metals (Gattullo et al., 2017). Plants may be utilized for phytoremediation of heavy metals from soil but they should be forbidden as diet and forage (Chandra and Kumar, 2017). There is opportunity to use the sewage sludge in farming according to the national standards (Cocarta et al., 2017). Drop in population of pathogenic microorganisms was noted during the composting of sewage sludge (Asses et al., 2018). The leaching of exchangeable heavy metals decreased considerably from the sewage sludge stabilized by fly ash (Zhang et al., 2017). Better hydraulic standards were observed in the compost made from a mixture of straw of maize, sewage sludge and biochar (Głąb et al., 2020).



**Fig. 1.** Preparation of Plant Samples



**Fig. 2.** Preparation of Soil & Sludge samples

**Table 1.** Concentration of heavy metals in different experimental setups

S. No.	Samples code	Samples	Concentration of Heavy Metals (mg/kg)			
			Pb	Cd	Hg	Zn
1.	S1	Only Sludge	1106.31	49.26	23.37	221.45
2.	S2	Only Soil	52.00	1.53	16.93	93.35
3.	S3	Sludge mixed with Soil	596.36	24.70	22.63	219.86
4.	S4	Dried Tomato plant grew on sludge	43.71	3.69	12.55	102.07
5.	S5	Dried Spinach plant grew on sludge	119.58	5.41	9.23	105.14
6.	S6	Dried Mustard plant grew on sludge	77.62	3.32	8.99	100.76
7.	S7	Sludge after Tomato was grown	1017.64	34.94	10.03	114.75
8.	S8	Sludge after Spinach was grown	904.59	28.07	9.63	113.21
9.	S9	Sludge after Mustard was grown	942.02	32.53	13.98	100.60
10.	S10	Dried Tomato plant grew on sludge mixed with soil	231.11	9.41	12.43	108.72
11.	S11	Dried Spinach plant grew on sludge mixed with soil	83.90	4.18	10.78	103.81
12.	S12	Dried Mustard plant grew on sludge mixed with soil	25.88	0.99	11.23	85.33
13.	S13	Sludge mixed with soil after Tomato was grown	361.46	12.86	21.23	110.15
14.	S14	Sludge mixed with soil after Spinach was grown	445.89	10.49	6.43	111.76
15.	S15	Sludge mixed with soil after Mustard was grown	442.12	9.68	6.57	112.03

Lead (Pb) was the major heavy metal that was present in the sludge collected from the UASB Mirzapur. It was about 1106.31 mg/kg which is quadruple the permissible level i.e. 300 mg/kg. Data revealed that lead concentration was more than permissible level, so the sludge was not suitable to be used as fertilizer in the soil, because the more the concentration of lead in the soil the more will be the uptake of lead by the plant and there is possibility that the lead may reach to the edible parts of the plant. When the three plant species were grown on the pure sludge then the accumulation of lead was maximum in tomato (119.56 mg/kg) followed by spinach (77.62 mg/kg) and the least accumulation was seen in the mustard plant. When the sludge was mixed with soil, the lead accumulation trends got changed. The lead accumulation trend was Mustard > Tomato > Spinach with values being 231.11, 83.90 & 25.88 mg/kg respectively.

Lead adversely affects morphology, growth and photosynthesis. (Nagajyoti et al., 2010). Lead also inhibits the seedling growth in rice (Mukherji, and Maitra, 1976), soya bean (Huang et al., 1974), maize (Miller et al., 1975), tomato, eggplant (Khan and Khan, 1983), barley (Stiborova et al., 1987) and few legumes (Sudhakar et al., 1992). Lead obstructs elongation of stem and root and expansion of leaf in barley (Juwarkar and Shende, 1986), *Allium* species (Gruenhage and Jager, 1985). Lead affects in different ways like radial thickness in roots of pea, lignification in cortical parenchyma of (Paivoke, 1983), proliferation effect in vascular plants during process of repair (Kaji et al., 1995) and chlorosis and reduction in growth in potted sugar beet (Hewilt, 1953), growth reduction in roots of carrot and lettuce (Baker, 1972). Lead also inhibits activities of enzymes (Sinha et al., 1988) imbalance of water, change in permeability of membrane and effects on mineral nutrition (Sharma and Dubey, 2005).

Cadmium (Cd) present in sludge collected from the UASB Mirzapur was about 49.26 mg/kg which was within the permissible level of 50 mg/kg. Considering only the low Cd concentration in sludge, it can be recycled as fertilizer, but the results in Table 1 suggest that some amount of cadmium is accumulated by all the three species. The highest amount, 5.41 mg/kg of cadmium was accumulated in the Tomato plant followed by 3.69 mg/kg by mustard

and least accumulation of 3.32 mg/kg in spinach plant. The similar tendency was seen in the plants when grown on sludge mixed with soil. The trend was Mustard > Tomato > Spinach with concentrations of 9.405, 4.18 & 0.99 mg/kg respectively. It could be possible that through the plant-heavy metal could also get accumulated in the edible parts of the plant which could be toxic for humans if the concentrations are more than the permissible level.

High concentration of Cadmium causes chlorosis, inhibition of growth, root tips browning and death. (Sanita, 1999; Wojcik, 2004; Mohanpuria, 2007; Guo, 2008). Cadmium interferes in the uptake, transport and use of the Ca, Mg, P, K and water (Das et al., 1997). Cadmium alters membrane functions (Fodor et al., 1995) and disturbs chloroplast metabolism. (De and Ziegler, 1993). Cadmium reduces the absorption and transport of nitrate (Hernandez et al., 1996).

The sludge collected from the UASB Mirzapur contained of mercury (Hg) having concentration of 23.37 mg/kg. Mercury in the sludge was more than the permissible level i.e. 17 mg/kg. The trend of accumulation of mercury in grown plants was Mustard > Tomato > Spinach with concentrations of 12.55, 9.23 & 8.99 mg/kg respectively. When the same species were grown on sludge mixed with soil, the trend got changed and now the trend was Mustard > Spinach > Tomato and the concentrations were 12.43, 11.23 & 10.78 mg/kg respectively.

Hg<sup>2+</sup> is predominant in agricultural soil (Han et al., 2006). Higher levels of Hg<sup>2+</sup> may cause injuries and physiological disorders. (Zhou et al., 2007), may induce closing of stomata (Zhang and Tyerman, 1996), interference of mitochondrial activity and so lead to the disturbance of cellular metabolism (Messer et al., 2005; Cargnelutti et al., 2006). Hg<sup>2+</sup> has capacity to be accumulated easily in aquatic and higher plants. (Kamal et al., 2004; Wang and Greger, 2004; Israr et al., 2006)

The sludge had Zinc concentration of 114.24 mg/kg which was below permissible level of 450 mg/kg. The trend of accumulation of zinc in grown plants was Tomato > Mustard > Spinach with concentrations of 105.14, 102.07 & 100.76 mg/kg respectively. When the same species were grown on sludge mixed with soil, the trend got changed and now the trend was Mustard > Tomato > Spinach and the concentrations were 108.72, 103.81 & 85.33 mg/kg respectively. Toxic levels of Zinc affect metabolism leads to growth retardation, chlorosis, senescence and limit the root and shoot growth and purplish-red color appearance in leaves (Choi et al., 1996; Ebbs and Kochian, 1997; Fontes and Cox, 1998; Lee et al., 1996)

## CONCLUSION

There are many technologies available to treat waste water but most of them are not feasible at all places. In Mirzapur, a power-starved district, the UASB technique of waste-water treatment was adopted. With rising standards of living, more heavy metals are being discharged in waste water. Almost all of the available waste-water treatment technologies do not treat heavy metals, so, is the case with the UASB. Under these circumstances, heavy metals are being discharged in sludge and the water that is released after the treatment. The results (Table 1) showed that the sludge which was collected from UASB Mirzapur contained heavy metals like Lead (Pb), Cadmium (Cd), Mercury (Hg) and Zinc (Zn) with concentrations of 1106.31, 49.26, 23.37 and 221.45 mg/kg respectively. The concentration of Lead, Cadmium, Mercury and Zinc in the soil were 52.00, 1.53, 16.93 and 93.35 mg/kg respectively. When the sludge got mixed with soil then concentrations of all the heavy metals got decreased in the mixture. The concentrations of Lead, Cadmium, Mercury and Zinc in the mixtures were 596.36, 24.70, 22.63 and 219.86 mg/kg respectively. The results of the present

study show that the trend of concentrations was  $Pb > Zn > Cd > Hg$  in the sludge. The trend of concentrations in the soil was of  $Zn > Pb > Hg > Cd$ . When the sludge was mixed with the soil the trend got changed and the trend was  $P > Zn > Cd > Hg$ . Three different plant species chosen for the study were *Basella alba* (Spinach), *Solanum lycopersicum* (Tomato) and *Brassica juncea* (Mustard). When these three different species were grown on the sludge then the trend of accumulation of heavy metals in *Brassica Juncea* (Mustard) was  $Zn > Pb > Hg > Cd$ . In *Solanum lycopersicum* (Tomato) the trend was  $Pb > Zn > Hg > Cd$  and in *Basella alba* (Spinach) the trend was  $Zn > Pb > Hg > Cd$ . When these three different species were grown on the sludge mixed with soil, the trend got changed and the trend of concentration of heavy metals and it became  $Pb > Zn > Hg > Cd$ . In *Solanum lycopersicum* (Tomato) the trend was  $Zn > Pb > Hg > Cd$  and in *Basella alba* (Spinach) the trend was  $Zn > Pb > Hg > Cd$ . The above given trend shows that Lead (Pb) and Zinc (Zn) were accumulated in maximum amounts in all the three species, whereas, the accumulation of Cadmium (Cd) and Mercury (Hg) was least in all the three species. Maximum percent reduction in Lead (Pb), Cadmium (Cd), Mercury (Hg) and Zinc (Zn) in the sludge mixed with soil was in *Solanum lycopersicum* (Tomato) (39.38%), *Brassica Juncea* (Mustard) (60.80%), *Basella alba* (Spinach) (71.58%) and *Solanum lycopersicum* (Tomato) (49.89%) respectively. So, *Solanum lycopersicum* (Tomato) can be utilized for the reduction of Lead (Pb) and Zinc (Zn) while *Brassica Juncea* (Mustard) and *Basella alba* (Spinach) can be utilized for the reduction of Cadmium (Cd) and Mercury (Hg) respectively. Overall the municipal sewage sludge of an USAB plant can be utilize for mixing it into the agricultural soil as manure and it this sewage sludge contains heavy metals like Lead (Pb), Cadmium (Cd), Mercury (Hg) and Zinc (Zn) then plants like *Solanum lycopersicum* (Tomato), *Brassica Juncea* (Mustard) and *Basella alba* (Spinach) etc. can be utilize to reduce their concentration to an extent where this sludge can be applied for agricultural practices safely.

Rising standard of living is bound to increase the use of heavy metals and its subsequent discharge in the waste-water. Since, none of the existing technologies is able to treat the heavy metals, the concentrations of heavy metals in sludge is likely to increase. There is evidence of the accumulation of heavy metals in plants; there are chances that heavy metals may get accumulated in the edible part of plants, thereby reaching the human beings through food chain. The accumulation of heavy metals in human beings can cause many problems for examples lead can result in a wide range of biological effects depending upon the level and duration of exposure. It shows its effects at the sub cellular level, as well as effects on the overall functioning of the body. It affects many biochemical processes; in particular, effects on haem synthesis have been studied in both adults and children. Cadmium reaches the liver through blood and bound to proteins and form complexes and transported to the kidneys and accumulates and damages the filtering mechanisms. This causes the excretion of essential proteins and sugars and further kidney damage. Exposure to mercury can diminish photosynthesis and other physiological activities of plants. Both organic and inorganic mercury induces loss of potassium, magnesium, and manganese with accumulation of iron. At the sub cellular level, the possible mechanisms through which heavy metals can damage comprise the blocking of important molecules (e.g., enzymes and polynucleotide). At high concentrations zinc adversely affect the activity of soil fauna. To control all these problems related to the accumulation of heavy metals in the plants there is a need to change to a technology having capability to remove heavy metals during the treatment of waste-water. The concentration of heavy metals should be checked in the sludge after treatment and then it should be sold as a fertilizer.

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## CONFLICT OF INTEREST

The authors declare that there is not any conflict of interests regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/ or falsification, double publication and/or submission, and redundancy has been completely observed by the authors.

## LIFE SCIENCE REPORTING

No life science threat was practiced in this research.

## REFERENCES

- Angino, E.E., Magnuson, L.M., Waugh, T.C., Galle, O.K. and Bredfeldt, J. (1970). Arsenic in detergents-possible danger and pollution hazard. *Sci*, 168; 389-392.
- Asses, N., Farhat, A., Cherif, S., Hamdi, M. and Bouallagui, H. (2018). Comparative study of sewage sludge co-composting with olive mill wastes or green residues: Process monitoring and agriculture value of the resulting composts. *Process Safety and Environment Protection*, 114; 25-35.
- Baker, W.G. (1972). Toxicity levels of mercury lead, copper and zinc in tissue culture systems of cauliflowers lettuce potato and carrot. *Can J Bot*, 50; 973-976.
- Bogusz, A., Oleszczuk, P. and Dobrowolski, R. (2017). Adsorption and desorption of heavy metals by the sewage sludge and biochar-amended soil. *Environ Geochem Health*, 41; 1663-1674.
- Bradford, W.I. (1997). Urban storm water pollutant loadings a statistical summary through. *JWPCF*, 49; 610-613.
- Cargnelutti, D., Tabaldi, L.A., Spanevello, R.M., Jucoski, G.O., Battisti, V., Redin, M., Linares, C.E.B., Dressler, V.L., Flores, M.M., Nicoloso, F.T., Morsch, V.M. and Schetinger, M.R.C. (2006). Mercury toxicity induces oxidative stress in growing cucumber seedlings. *Chemosph*, 65; 999-1106.
- Chandra, R. and Kumar, V.(2017). Phytoextraction of heavy metals by potential native plants and their microscopic observation of root growing on stabilised distillery sludge as a prospective tool for in situ phytoremediation of industrial waste. *Environ Sci Pollut Res*, 24; 2605–2619.
- Chen, Z., Zhao, Y., Li, Q. and Qiao J. et. al. (2009). Heavy metal contents and chemical speciations in sewage-irrigated soils from the eastern suburb of Beijing, China. *Journal of food, agriculture & environment*, 7(3-42); 690-695.
- Choi, J.M, Pak, C.H. and Lee, C.W. (1996). Micronutrient toxicity in French marigold. *J Plant Nutri*, 19; 901-916.
- Chu, S., Wu, D., Liang, L. L., Zhong, F., Hu, Y., Hu, X., Lai, C. and Zeng, S. (2017). Municipal sewage sludge compost promotes *Mangifera persiciforma* tree growth with no risk of heavy metal contamination of soil. *Scientific Reports*, 7; 13408. <https://doi.org/10.1038/s41598-017-13895-y>.
- Clarke, R., Peyton, D., Healy, M.G., Fenton, O., and Cummins, E. (2016). A quantitative risk assessment for metals in surface water following the application of biosolids to grassland. *Sci. Total Environ*, 566-567; 102–112. <https://doi.org/10.1016/j. scitotenv.2016.05.092>.

- Cocarta, D.M., Subtirelu, V. R. and Badea, A. (2017). Effect of sewage sludge application on wheat crop Productivity and heavy metal accumulation in soil and wheat grain. *Environmental Engineering and Management Journal*, 16(5); 1093-1100.
- Cunningham, R.P. (1997). DNA repair: caretakers of the genome. *Curr Biol*, 7; 576-579.
- Das, P., Samantaray, S. and Rout, G.R. (1997). Studies on cadmium toxicity in plants: a review. *Environ Pollut*, 98; 29-36.
- De Filippis, L.F. and Ziegler, H., (1993). Effect of sublethal concentrations of zinc, cadmium and mercury on the photosynthetic carbon reduction cycle of *Euglena*. *J Plant Physiol*, 142; 167-172.
- Ebrahim, M. E., Sulaiman, A. A., Ahmed, F. E.B., Khaled, F. F., Mostafa, A. T., Abd, E. L. H., Gamal, A. E S., and Mohamed, T. A. (2018). Evaluation of the potential of sewage sludge as a valuable fertilizer for wheat (*Triticum aestivum* L.) crops. *Environmental Science and Pollution Research*, 26; 392-401.
- Ebbs, S.D. and Kochian, L.V. (1997). Toxicity of zinc and copper to Brassica species: implications for Phytoremediation. *J Environ Qual*, 26; 776-781.
- Fernandes, J.C. and Henriques, F.S. (1991). Biochemical, physiological and structural effects of excess copper in plants. *Bot Rev*, 57; 247-273.
- Fodor, A., Szabo-Nagy, A. Erdei, L. (1995). The effects of cadmium on the fluidity and H<sup>+</sup>-ATPase activity of plasma membrane from sunflower and wheat roots. *J Plant Physiol*, 14; 787-792.
- Fontes, R.L.S. and Cox, F.R. (1998). Zinc toxicity in soybean grown at high iron concentration in nutrient solution. *J Plant Nutri*, 21; 1723-1730.
- Gattullo, C. E., Mininni, M., Parente, A., Montesano, F. F., Allegretta, I. and Terzano, R. (2017). Effects of municipal solid waste- and sewage sludge-compost-based growing media on the yield and heavy metal content of four lettuce cultivars. *Environ Sci Pollut Res*, 24; 25406–25415.
- George, F. A. and Snyder, J. C. (2007). Accumulation of heavy metals in plants and potential phytoremediation of lead by potato, *Solanum tuberosum* L., *J Environ. Sci Health Part A*, 42(6); 811-816.
- Głąb, T., Żabińska, A., Sadowska, U., Gondek, K., Kopeć, M., Hersztek, M.M., Sylwester Tabor, S., and Tarkowska, J.S.(2020). Fertilization effects of compost produced from maize, sewage sludge and biochar on soil water retention and chemical properties. *Soil & Tillage Research*, 197; 104493. <https://doi.org/10.1016/j.still.2019.104493>.
- Gruenhagen, L. and Jager, I.I.J. (1985). Effect of heavy metals on growth and heavy metals content of *Allium Porrum* and *Pisum sativum*. *Angew Bot*, 59; 11-28.
- Guo, J., Dai, X., Xu, W. and Ma, M. (2008). Over expressing GSHI and AsPCSI simultaneously increases the tolerance and accumulation of cadmium and arsenic in *Arabidopsis thaliana*. *Chemosphere*, 72; 1020-1026.
- Han, F.X., Su, Y., Monts, D.L., Waggoner, A.C. and Plodinec, J.M. (2006). Binding distribution and plant uptake of mercury in a soil from Oak Ridge, Tennessee, USA. *Sci Total Env*, 368; 753-768.
- Hawkes, J. S. (1997). Heavy metals. *J Chem Edu*, 74;1369-1374.
- Hei, L., Lee, C.C., Wang, H., Lin, X.Y., Chen, X.H., and Wu, Q.T. (2016). Using a high biomass plant *Pennisetum hybridum* to phyto-treat fresh municipal sewage sludge. *Bioresour. Technol*, 217; 252–256.
- Hernandez, L.E., Carpena-Ruiz, R. and Garate, A. (1996). Alterations in the mineral nutrition of pea seedlings exposed to cadmium. *J Plant Nutr*, 19; 1581-1598.
- Hewilt, E.J. (1953). Metal inter-relationships in plant nutrition. *J Exp Bot*, 4; 59-64.
- Huang, C.V., Bazzaz, F.A. and Venderhoef, L.N. (1974). The inhibition of soya bean metabolism by cadmium and lead. *Plant Physiol*, 34; 122-124.
- Israr, M., Sahi, S., Datta, R. and Sarkar, D. (2006). Bioaccumulation and physiological effects of mercury in *Sesbania drummonii*. *Chemosphere*, 65; 591-598.
- Jakubus, M. and Czekala, J. (2001). Heavy Metal Speciation in Sewage Sludge. *Polish Journal of Environmental Studies*, 10(4); 245-250.
- Jarosław, G. and Barbara, G. (2012). Mobility of heavy metals in municipal sewage sludge from different throughput sewage treatment plants. *Pol. J. Environ. Stud.* 21(6); 1603-1611.



- Juwarkar, A.S. and Shende, G.B. (1986). Interaction of Cd-Pb effect on growth yield and content of Cd, Pb in barley. *Ind J Environ Heal*, 28; 235-243.
- Kaji, T., Suzuki, M., Yamamoto, C., Mishima, A., Sakamoto, M. and Kozuka, H. (1995). Severe damage of cultured vascular endothelial cell monolayer after simultaneous exposure to cadmium and lead. *Arch Environ Contam Toxicol*, 28; 168-172.
- Kamal, M., Ghaly, A.E., Mahmouda, N. and Cote, R. (2004). Phytoaccumulation of heavy metals by aquatic plants. *Environ Intern*, 29(8); 1029-1039.
- Kasprzak, K.S. (1995). Possible role of oxidative damage in metal-induced carcinogenesis. *Cancer Invest*, 13; 411-430.
- Khan, S. and Khan, N.N. (1983). Influence of lead and cadmium on growth and nutrient concentration of tomato (*Lycopersicon esculentum*) and eggplant (*Solanum melongena*). *Plant Soil*, 74; 387-394.
- Kominko H., Gorazda K., and Wzorek Z. (2019). Potentiality of sewage sludge-based organo-mineral fertilizer production in Poland considering nutrient value, heavy metal content and phytotoxicity for rapeseed crops. *Journal of Environmental Management*; 248. <https://doi.org/10.1016/j.jenvman.2019.109283>.
- Lee, C.W., Choi, J.M. and Pak, C.H. (1996). Micronutrient toxicity in seed geranium (*Pelargonium hortorum* Bailey). *J Am Soc Horti Sci*, 121; 77-82.
- Liu, L., Wang, S., Guo, X. and Wang H. (2019). Comparison of the effects of different maturity composts on soil nutrient, plant growth and heavy metal mobility in the contaminated soil. *Journal of Environmental Management*; 250. <https://doi.org/10.1016/j.jenvman.2019.109525>.
- Liu, K., Li, C., Tang, S., Shang, G., Yu, F. and Li, Y. (2020). Heavy metal concentration, potential ecological risk assessment and enzyme activity in soils affected by a lead-zinc tailing spill in Guangxi, China. *Chemosphere*, 251. <https://doi.org/10.1016/j.chemosphere.2020.126415>.
- Lixandru, B., Viorel, P., Elisabeta, P.L., Dragomir, N., Pricop, A., Mășu, S., Morariu, F. and Popescu, D. (2010). Research Regarding the Accumulation in Soybeans of Heavy Metals from Anaerobic Composted Sewage Sludge Used as Organic Fertilizer. *Animal Science and Biotechnologies*, 43(2); 93-97.
- Messer, R.L., Lockwood, P.E., Tseng, W.Y., Edwards, K., Shaw, M., Caughman, G.B., Lewis, J.B. and Wataha, J.C., (2005). Mercury (II) alters mitochondrial activity of monocytes at sublethal doses via oxidative stress mechanisms. *J Biomed Mat Res B*, 75; 257-263.
- Miller, J.E., Hassete, J.J. and Koppe, D.E. (1975). Interaction of lead and cadmium of electron energy transfer reaction in corn mitochondria. *Physiol Plant*, 28; 166-171.
- Misra, S.G. and Mani, D. (Eds.) (1991). *Soil pollution*. (New Delhi, India: Ashish Publishing House)
- Mohanpuria, P., Rana, N.K. and Yadav, S.K. (2007). Cadmium induced oxidative stress influence on glutathione metabolic genes of *Camellia sinensis* (L.). *O Kuntze. Environ Toxicol*, 22; 368-374.
- Monu, A. Bala, K., Shweta, R., Anchal, R., Barinder, K. and Neeraj, M. (2008). Heavy metal accumulation in vegetables irrigated with water from different sources. *Food Chemistry*, 111; 811-815.
- Mukherji, S. and Maitra, P. (1976). Toxic effects of lead growth and metabolism of germinating rice (*Oryza sativa* L.) seeds mitosis of onion (*Allium cepa*) root tip cells. *Ind J Exp Biol*, 14; 519-521.
- Nagajyoti, P. C., Lee, K. D. and Sreekanth, T. V. M. (2010). Heavy metals, occurrence and toxicity for plants: a review. *Environ Chem Lett*, 8; 199-216.
- Nahar, N. and Hossen, M. S. (2020). Influence of sewage sludge application on soil properties, carrot growth and heavy metal uptake. *Communications in Soil Science and Plant Analysis*, 52. <https://doi.org/10.1080/00103624.2020.1836201>.
- Paivoke, H. (1983). The short term effect of zinc on growth anatomy and acid phosphate activity of pea seedlings. *Ann Bot*, 20; 307-30.
- Peplow, D. (1999). Environmental impacts of mining in Eastern Washington. Center for Water and Watershed studies fact sheet, University of Washington, Seattle.

- Reeves, R.D. and Baker, A.J.M. (2000). Metal-accumulating plants. (In Raskin I. and Ensley B.D. (Eds.), *Phytoremediation of toxic metals: using plants to clean up the environment* (pp. 193-229). New York: Wiley.)
- Samara, E., Matsi, T. and Balidakis, A. (2017). Soil application of sewage sludge stabilized with steelmaking slag and its effect on soil properties and wheat growth. *Waste Management*, 68; 378-387.
- Sanita, D.T.L. and Gabbrielli, R. (1999). Response to cadmium in higher plants. *Environ Exp Bot*, 41; 105-130.
- Sharma, P. and Dubey, R.S. (2005). Lead toxicity in plants. *Braz J Plant Physiol*, 17; 35-52.
- Sinha, S.K., Srinivastava, H.S. and Mishra, S.N. (1988). Nitrate assimilation in intact and excised maize leaves in the presence of lead. *Bull Environ Cont Toxi*, 41; 419-422.
- Sinha, S.K., Srinivastava, H.S. and Mishra, S.N. (1988). Effect of lead on nitrate reductase activity and nitrate assimilation in pea leaves. *Bot Pollu*, 57; 457-463.
- Stadtman, E.R. (1993). Oxidation of free amino acids and amino acid residues in proteins by radiolysis and by metal-catalysed reactions. *Annu Rev Biochem*, 62; 797-821.
- Stiborova, M., Pitrichova, M. and Brezinova, A. (1987). Effect of heavy metal ions in growth and biochemical characteristic of photosynthesis of barley and maize seedlings. *Biol Plant*, 29; 453-467.
- Sudhakar, C., Simalabai, L. and Veeranjaveyuler, K. (1992). Lead tolerance of certain legume species grown on lead or tailing. *Agri Eco Environ*, 41; 253-261.
- Tontti, T., Poutiainen, H., Heinonen-Tanski, H. (2017). Efficiently treated sewage sludge supplemented with nitrogen and potassium is a good fertilizer for cereals. *Land Degrad. Dev*, 28 (2), 742-751.
- Wang, Y. and Greger, M. (2004). Clonal differences in mercury tolerance, accumulation, and distribution in willow. *J Environ Qual*, 33; 1779-1785.
- Wojcik, M. and Tukiendorf, A. (2004). Phytochelatin synthesis and cadmium localization in wild type of *Arabidopsis thaliana*. *Plant Growth Regul*, 44; 71-80.
- Wong, J.W.C. (1996). Heavy metal contents in vegetables and market garden soils in Hong Kong. *Environ Technol*, 17: 407-414.
- Yamamoto, F. and Kozlowski, T.T. (1987). Effect of flooding, tilting of stem, and ethrel application on growth, stem anatomy, and ethylene production of *Acer platanoides* seedlings. *Scand J For Res*, 2; 141-156.
- Zhang, H., Ma, G., Sun, K. and Li, H. (2017). Effect of alkaline material on phytotoxicity and bioavailability of Cu, Cd, Pb and Zn in stabilized sewage sludge. *Environmental Technology*, 39; 2168-2177.
- Zhang, W.H. and Tyerman, S.D. (1999). Inhibition of water channels by HgCl<sub>2</sub> in intact wheat root cells. *Plant Physiol*, 120; 849-857.
- Zhou, D., Liu, D., Gao, F., Li, M. and Luo, X. (2017). Effects of Biochar-Derived Sewage Sludge on Heavy Metal Adsorption and Immobilization in Soils. *Int. J. Environ. Res. Public Health*, 14(7); 681. <https://doi.org/10.3390/ijerph14070681>.
- Zhou, Z.S., Huang, S.Q., Guo, K., Mehta, S.K., Zhang, P.C. and Yang, Z.M. (2007). Metabolic adaptations to mercury-induced oxidative stress in roots of *Medicago sativa* L. *J Inorg Biochem*, 101;1-9.

